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REPELLENCY AND TOXICITY OF FOUR ESSENTIAL OILS TO *SITOPHILUS ORYZAE* L. (COLEOPTERA: CURCULIONIDAE)

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Abstract: The essential oils of the leaves of *Cymbopogon citratus, Cymbopogon nardus, Cinnamomum zeylanicum* and rhizome of *Alpinia calcarata* grown in Sri Lanka were tested for repellent activity, fumigant toxicity and contact toxicity against *Sitophilus oryzae*. The major components of the essential oils were geraniol in *C. nardus*, citral *a* and *b* in *C. citratus*, eugenol in *C. zeylanicum* and 1,8-cineol in *A. calcarata*. In a dual choice repellency test, repellency to *S. oryzae* increased with increasing dose of each oil except *C. zeylanicum* at a dose of 1 mg. *Cymbopogon citratus* was the most toxic oil to *S. oryzae* during the fumigant toxicity test with an LC_{50} value of 0:035 g/l. Adults of *S. oryzae* were equally susceptible to the fumigant toxicity of *C. nardus* and *C. zeylanicum* at 0.1 g/l level. Furthermore, *S. oryzae* adults were tolerant to the contact (0.15 g/m^2) and fumigant (0.1 g/l) activities of *A. calcarata* oil and the mortality of the test insects was not significantly different from the controls.

Key words: essential oils, rice, *Sitophilus oryzae*, stored grain

INTRODUCTION

Sitophilus oryzae L. is one of the major pests of stored cereals and the predominant pest of stored rice. The control of insect pests in storage is largely based on synthetic insecticides and fumigants (pirimiphos methyl and phosphine) which have led to the development of insecticide resistant strains, increasing cost of application, lethal effects on non-target organisms in addition to direct toxicity to users.[1-3] Thus repellents, fumigants, feeding deterrents and insecticides of natural origin obtained from the respective regions are rational alternatives to synthetic insecticides.[4,5] Certain essential oils, on account of their volatile nature and other traditional uses, offer possibilities for their use as effective repellents and toxicants against stored grain pests.[6-8] In many Asian and African countries it is an age-old practice to mix plant parts with grain to manage stored grain pests.[9,10] Mixing of plant leaves of *Vitex negundo*, *Ocimum sanctum*, *Eucalyptus terreticornis* and *Citrus* sp., which contain essential oils with stored paddy at 1 % (w/w), effectively controlled primary and secondary insect pests including *S. oryzae*.[11] The bark extract of *Melia*

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toosendan was found to be repellent and toxic to *S. oryzae*.[8] The essential oil from *Labiatae* sp. at concentrations varying from 1.4 to 4.5 µl/ l induced 90 % mortality in stored product beetles including *S. oryzae* after 24 h of fumigation.[12] Bioactivity of essential oils of *Evodia rutaecarpa*, nutmeg seeds and *Elletaria cardamomum* have been reported to have repellent and toxic activities against *S. zeamais*.[5-7]

In Sri Lanka, farmers use essential oil-bearing plants, which release terpenes for the control of stored grain pests. *Cymbopogon nardus* Rendle, *Cymbopogon citratus* (DC.) Stapf., *Cinnamomum zeylanicum* Blume and *Alpinia calcarata* Rosc. are some of the essential oil-bearing plants grown in Sri Lanka.

C. citratus (lemongrass) and *C. nardus* (citronella) belong to the family Poaceae (Graminae) and yield essential oils which are mainly used in the spice and essential oil industry. *C. citratus* yields an essential oil with >70 % of citral *a* and *b*. The essential oil is used to combat mosquitoes and houseflies at a dose of 30 and 7.5 µg per insect.[13] Powdered C. citratus has been used as a repellent against *Callasobruchus* sp.[14] Geraniol (19 %) is the major component of the essential oil of *C. nardus*.[15] The essential oil of *C. nardus* has been shown to have a repellent activity against *Tribolium castaneum, Sitotroga cerealella, Callasobruchus chinensis* and many other stored grain insect pests.[16] The essential oil of C. nardus was found to have toxic and repellent actions on *C. maculatus*.[17,18] *C. zeylanicum* Blume (Lauraceae) (cinnamon) leaf oil contains 77 % of eugenol.[19] Eugenol was shown to have contact toxicity against *S. zeamais* (LD_{50} 30 µg/ mg insect).[20] *A. calcarata* Rosc. (Zingiberaceae) (S. Heenaraththa) rhizomes are used in indigenous medicine. Tewari *et al.* have reported the presence of 42 % of 1,8-cineol in the rhizome essential oil and repellent activity of the essential oil was tested against *Periplanata americana*.[21,22] This study was undertaken to evaluate the bioefficacy of the essential oils from the leaves of *C. citratus, C. nardus, C. zeylanicum* and rhizomes of *A. calcarata* on *S. oryzae* with a view to develop an environmentally safer, effective and economical control method.

METHODS AND MATERIALS

Insects: S. oryzae was obtained from laboratory cultures maintained at 28 ± 3 °C, 70 – 80 % r.h. and a photoperiod of 12:12 (L:D). One-week-old adult insects were used for all the bioassays.

Essential oils: The essential oils of *C. zeylanicum, C. nardus* and *C. citratus* leaves were purchased from the Industrial Technology Institute, Hendrik and Sons and EOAS Organics Ltd., Colombo, respectively. Rhizomes of *A. calcarata* were cut into pieces, air-dried and steam distillated for 3 h. The distillate was extracted into dichloromethane and concentrated on a rotary evaporator (R-114 and B-180, BÜCHI, Labortechnik AG, Flawil, Switzerland) at 35 °C. Remaining solvent was evaporated under a dry nitrogen stream and residue stored at 4 – 5 °C in sealed

glass vials. Different dilutions of the essential oils were made using ethanol in all the experiments.

Gas chromatographic (GC) analysis of the essential oils: The chemical constituents of each essential oil used for bioassays in the present study were analyzed on a GC having the following specifications (HP5890 series II chromatograph, Hewlett Packard, Palo Alto, CA, USA with FID and DBwax capillary column, J & W Scientific, Folsom, CA, USA, 30 m x 0.25 mm; 0.25 μm film thickness). The column was programmed as follows; 40 °C (0 min.), 40 °C to 210 °C at 5 °C/min, 210 °C (10 min.) with Helium carrier gas (1 ml/min). The injector and detector temperatures were 220 °C and 270 °C respectively, and 1 μl of the oil solution in CH_2Cl_2 (2 mg/ml) was injected and the constituents were analysed and compared with published data.[15,19]

Repellent activity: A "Y" shaped olfactometer with 3 connected glass tubes (10 cm long, 1 cm diameter) with an opening at the intersection of the 3 arms for the vacuum pump was used as the olfactometer. The opening on the intersection of the arms facilitated the air circulation in the olfactometer.[23] The ends of the two tubes of the olfactometer were connected with perforated, plastic, transparent, wide mouthed bottles (250 ml) through the lids and the other end of the tube was used to introduce insects. Two Whatman no. 1 filter papers (2.5 cm x 2.5 cm), one treated with a known amount of essential oil and the other treated with equal amount of ethanol were hung separately, after air-drying for 10 minutes, in the middle of the bottles connected to the two tubes using metal wires. The olfactometer was placed horizontally on a white background in daylight. After switching on the vacuum pump, ten test insects were introduced into the olfactometer. The number of insects that moved into the essential oil treated and ethanol treated bottles within 30 minutes were recorded. Five doses of *C. citratus* (10 - 150 mg), C. nardus (0.5 – 7.5 mg), *C. zeylanicum* (1.0 – 10.0 mg) and *A. calcarata* (0.5 – 7.5 mg) were tested separately and each dose was replicated 5 times. Placement of the essential oil treated and the ethanol treated filter papers were interchanged randomly in subsequent replicates. At each trial, the olfactometer was washed thoroughly with a detergent and dried in an oven. This assay was carried out between 07.00 and 10.00 h. The mean number of insects that responded to the two treatments at each dose was compared by Chi Square test.

Fumigant toxicity: Whatman no. 1 filter paper discs (1 cm diameter), each impregnated with essential oils dissolved in ethanol to give concentrations of 20.0, 30.0, 40.0, 50.0, 60.0 and 100.0 mg/l air for *C. citratus*, 10.0, 50.0, 100.0, 150.0 and 200.0 mg/l air for *C. nardus,* 50.0, 100.0, 250.0, 500.0 and 750.0 mg/l air for *C. zeylanicum* and 100.0, 200.0, 300.0, 400.0 and 500.0 mg/l air for *A. calcarata* were used separately. Each disc was placed on the underside of the screw cap of each glass bottle (7 ml) and the solvent was allowed to evaporate for 10 min. The neck of each bottle containing 10 insects was blocked with metal mesh (1 cm diameter).

The bottles were incubated at 28 ± 3 °C for 48 h in the dark. A similar procedure was carried out with ethanol and untreated samples were used as the control. Each treatment and control was replicated 5 times. At the end of the 48 h fumigant exposure period mortality was recorded. The mean mortality for each essential oil concentration was compared using ANOVA and Tukey's pair-wise comparison test. The LC50 values were determined by Probit Analysis using a computer package.

Contact toxicity: Glass bottles similar to those used in fumigant toxicity test were used (20 cm^2 area of the inner surface). Different doses of the test essential oils dissolved in ethanol were applied onto the inner surface of the bottles to give the concentrations of 3.7, 7.5, 10.0, 15.0, 20.0 µg/cm^2 of *C. citratus,* 6.0, 9.0, 12.0, 15.0, 20.0 µg/cm^2 of *C. nardus,* 2.5, 5.0, 7.5, 10.0, 12.5, 15.0 µg/cm^2 of *C. zeylanicum* and 15.0, 30.0, 45.0, 60.0, 75.0, 90.0 µg/cm^2 of *A. calcarata*. Ethanol was evaporated under a dry N_2 stream and 10 insects were introduced into each bottle. There after the procedure followed was similar to the fumigant toxicity assay.

Fumigant and contact toxicity tests were carried out with Actellic® (Pirimiphos methyl) as the synthetic pesticide and the LC50 values for comparison were obtained using probit analysis.

RESULTS

The main constituents identified by the respective relative retention times in the essential oils are listed in Table 1. Citral *a* and *b* (77.8%) were the main volatiles in *C. citratus*. Geraniol and limonene in *C. nardus* and eugenol in *C. zeylanicum* leaf were the major constituents of the respective oil. The essential oil of *A. calcarata* contained 1,8-cineole as the major constituent and the camphoraceous odour of the essential oil was due to 1,8-cineol and camphor.

S. oryzae were unable to recognize the oil treated bottle when given a choice between the essential oil treatment and control at 10 and 25 mg of *C. citratus*, 0.5 mg of *C. nardus* and 0.5 and 1.0 mg of *A. calcarata* (Table 2). However at higher doses of each oil, a significant number of test insects moved away from the treated bottles. Although, at the dose of 1.0 mg of *C. zeylanicum,* significantly higher number of insects moved into the treated bottle compared to the control, the results indicate that *C. zeylanicum* oil acts as a repellent at higher doses (> 5 mg).

Table 3 shows the fumigant and contact effects of each essential oil at 100.0 mg/l and 15.0 µg/cm^2 concentrations, respectively. *S. oryzae,* when exposed to fumigants of each essential oil at 100.0 mg/l concentration the highest susceptibility was shown for *C. citratus* oil. At similar concentrations, *C. nardus* and *C. zeylanicum* oils were not significantly different in their fumigant activitiy ($p<0.05$). The essential oil of *A. calcarata* showed the lowest fumigant activity and the response of insects was not significantly different from the control and the

ethanol treated samples ($p<0.05$). The lowest and the highest LC_{50} values of 35 mg/l and 367 mg/l were obtained for *C. citratus* and *A. calcarata* respectively during the fumigant toxicity assay.

Table 1: Composition of major constituents of essential oils found in four plant species.

Plant species	Major constituents (%)*
Cymbopogon citratus	citral *a* (46.2)
	citral *b* (31.6)
	geraniol (3.6)
	geranyl acetate (1.3)
Cymbopogon nardus	geraniol (17.7)
	limonene (9.8)
	camphene (8.6)
	barneol (7.5)
	methyl isoeugenol (6.8)
	citronellal (4.3)
Cinnamomum zeylanicum	eugenol (74.2)
	β-caryphyllene (3.5)
	benzyl benzoate (2.8)
	cinnamaldehyde (2.7)
	linalool (2.2)
Alpinia calcarata	1,8-cineole (49.9)
	fenchylacetate (7.6)
	camphor (6.4)
	β-pinene (6.8)
	camphene (4.4)

* based on peak area

Contact toxicity of the four oils was compared at 15.0 µg/cm^2 concentration (Table 3). The results revealed that *C. zeylanicum* has the highest mortality (100 %) after 48 h of exposure. At a dose of 15.0 µg/cm^2, *C. nardus* and *C. citratus* showed 70 % and 46 % mortality whereas *A. calcarata* oil did not show any contact toxic effect against *S. oryzae* at these concentrations. Based on LC_{50} values of the four essential oils, *S. oryzae* showed the highest susceptibility to *C. zeylanicum* oil. LC_{50} values for the essential oils of *C. citratus* and *C. nardus* were not significantly different from each other ($p>0.05$). In contrast to contact toxicity and fumigant toxicity of essential oils tested, *S. oryzae* was highly susceptible for pirimiphos methyl (Table 4).

DISCUSSION

The chemical constituents of the essential oils of *C. citratus, C. nardus, C. zeylanicum* and *A. calcarata* grown in Sri Lanka are similar to those reported previously.[15,19,22]

Table 2: Response of *S. oryzae* to essential oil treated and ethanol treated bottles during olfactometer bioassay.

Essential oil (plant spp.)	Dose (mg) E. oil treated	% Response ± S. E.§		Σ^2_1	P value*
		EtOH treated			
C. citratus	10	34 ± 2.4	34 ± 10.2	0	<0.05
	25	30 ± 6.2	38 ± 3.5	1.0	<0.05
	75	26 ± 4.9	58 ± 7.1	12.2**	>0.05
	100	14 ± 4.9	78 ± 4.4	44.5**	>0.05
	150	10 ± 5.3	74 ± 10.7	48.7**	>0.05
C. nardus	0.5	28 ± 5.8	28 ± 4.8	0	<0.05
	1.0	28 ± 1.9	50 ± 5.4	6.2**	>0.05
	2.5	14 ± 6.7	28 ± 5.8	4.6**	>0.05
	5.0	12 ± 1.9	70 ± 5.4	41.0**	>0.05
	7.5	5 ± 2.5	42 ± 5.6	29.1**	>0.05
C. zeylanicum	1.0	40 ± 0	27 ± 2.5	13.3**	>0.05
	2.0	33 ± 2.2	25 ± 5.7	1.1	<0.05
	5.0	30 ± 5.1	42.5 ± 9.2	2.1	<0.05
	7.5	15 ± 5.7	55 ± 5.7	22.8**	>0.05
	10.0	15 ± 4.4	55 ± 5.7	22.8**	>0.05
A. calcarata	0.5	40 ± 5.4	42 ± 3.7	0	<0.05
	1.0	30 ± 4.4	44 ± 3.9	2.6	<0.05
	2.5	16 ± 3.9	56 ± 7.4	22.2**	>0.05
	5.0	14 ± 4.9	52 ± 5.8	21.8**	>0.05
	7.5	12 ± 1.9	56 ± 2.4	28.4**	>0.05

*p< 0.05, 3.84 **significant at 5% (Chi square test)

§Ten insects were used in each replicate and mean of 5 replicates

In the olfactometer bioassay, *S. oryzae* showed a decrease in response with increasing dose of oil, except to *C. zeylanicum* oil at the dose of 1 mg. Eugenol, linalool, β-caryphyllene are some of the major constituents in *C. zeylanicum* leaf

oil.[15] Eugenol and linalool are behaviour modifying chemicals of many insects.[24] This could be the reason for the possible attractant effect of *C. zeylanicum* oil at 1 mg. A two-choice test similar to the present study, was carried out with water extracts of *Salvia officinalis, Artemisia absimthium, Sambucus nigra, Matricaria chamomilla* and *Anthum graveolous* where less than 30% response was obtained for each extract at 15 g herbs/ 200 ml water using *S. granaries.*[2] Jembere *et al.* have demonstrated the repellent effect of *Ocimum kilimandscharicum* at 0.3 g/ 250 g of wheat against *S. zeamais.*[25] The essential oil of *Evodia rutecarpa* had a higher repellent effect to *Tribolium castaneum* than to *S. zeamais* during the treated repellency filter paper disc test.[5]

Table 3: Mortality of *S. oryzae* following the exposure to fumigant and contact effects of four essential oils.

Essential oil	% Mortality ± S. E.*	
(Plant spp.)	Fumigant (100 mg/l)	Contact (15.0 µg/cm^2)
C. citratus	100 ± 0^a	46.0 ± 12.8^a
C. nardus	65.0 ± 6.7^b	70.0 ± 7.0^b
C. zeylanicum	58.3 ± 8.7^b	100 ± 0^c
A. calcarata	8.3 ± 3.0^c	0^d
Control	10.0 ± 0.8^c	4.0 ± 2.4^e
Ethanol	11.0 ± 1.2^c	4.0 ± 2.4^e

* Mean of 5 replicates, means followed by similar letters within the column are not significantly different (ANOVA and Tukey's pair-wise comparison test)

Table 4: LC_{50} values of *S. oryzae* for fumigant and contact toxicity to four essential oils.

Essential oil	LC_{50} value*	
(Plant spp.)	Fumigant (mg/l)	Contact (µg/cm^2)
C. citratus	35	11.5
C. nardus	82	18.7
C. zeylanicum	70	3.6
A. calcarata	367	40.0
Pirimiphos methyl	$3.2x10^{-1}$	$2.5x10^{-1}$

*48 hours exposure period

According to the present study, essential oils of *C. citratus* and *C. zeylanicum* are the most potent fumigant and contact toxicant respectively. *C. citratus* oil contains linalool and linalyl acetate in addition to citral *a* and *b*. It has been observed that monoterpene aldehydes have the highest fumigant effect against *T. castaneum*.[26] *Mentha citrata* oil containing linalool and linalyl acetate has shown a significant fumigant effect to *S. oryzae*.[27] Hence, it is likely that the high fumigant effect of essential oils of *C. citratus* could be due to the presence of citral *a* and *b*, linalool and linalyl acetate. Eugenol, a phenyl propionoid, is the major constituent of *C. zeylanicum* leaf oil and topical application of phenols was more toxic to *T. castaneum* than other saturated alcohols.[19,26] These findings are in agreement with the results obtained for the contact toxicity test in the present study.

S. oryzae adults were more tolerant to the fumigant and the contact effect of *A. calcarata* oil. The major constituent, 1,8-cineol, has been reported to have insecticidal properties against stored product beetles such as *S. oryzae, Lasioderma serricorne* (F.) and *Stegobium paniceum* (L.). The adults of *T. castaneum* were susceptible to both contact and fumigant toxicities of 1,8-cineol and LD_{50} values of 108.4 µg/mg of body weight of adult insect and 1.52 µg/l air were obtained respectively.[28] The low persistence rate and significant loss of toxicity of 1,8-cineol have also been observed.[29] Therefore, for the control of *S. oryzae* higher concentration of A. calcarata oil is required in toxicity assays. However, accumulation of high concentrations of terpenes in rice could affect the quality of the grain and their consumption.[30]

Contact and fumigant toxicities of essential oils to stored product beetles have been studied extensively. Cinnamaldehyde, the main constituent of cinnamon bark oil had similar fumigant and contact toxic effects on both *T. castaneum* and *S. zeamais*.[31] Cardamom and nutmeg oils were generally more effective contact poisons and fumigants against adults of *S. zeamais* than those of *T. castaneum*.[6,7] The toxicity and ovicidal activity of *C. nardus* oil were tested against adults and the eggs of *Callasobruchus maculatus* and the eggs were more susceptible to the test oil than the adults.[17]

In the present study, LC_{50} values for pirimiphos methyl were determined for *S. oryzae* during fumigant and contact toxicity assays. The results indicated that pirimiphos methyl is more effective than the essential oils tested. However, in view of the mammalian toxicity and development of environmental friendly products, these essential oils will still be prefered.

The present study on the biological activity of tested essential oils revealed the potential of the essential oils as a pest control agent of stored grain. The essential oils of *C. citratus* and *C. zeylanicum* showed the highest potential to be used as a fumigant and as a contact toxicant. *C. nardus* gave the highest repellent activity. Hence, these studies suggest that *C. citratus, C. nardus, C. zeylanicum* and *A.*

calcarata oils could be developed as potential grain protectants against adults of *S. oryzae*. However, there is a need to asses the cost-effectiveness and feasibility of using these essential oils on a large scale as grain protectants. Isolation and identification of effective compounds from the essential oils having toxic properties is necessary before considering the commercial application.

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